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CHEMISTRY, BIOCHEMISTRY, PHARMACOLOGY, AND TOXICOLOGY OF CS AND SYNTHESIS OF ITS NOVEL ANALOGS

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14. ABSTRACT
Of the potential five groups of less-than-lethal riot control and crowd management agents, three groups, namely ω-chloroacetophenones (CN), benzylidene malononitriles (CS-type), and dibenzooxazepines (CR) have found frequent use. Of the three groups, chlorobenzylidene malononitrile (CS) is the most popularly used non-lethal agent in riot control situations and military exercises. With a view to improve its properties and enhance its effectiveness, 15 new CS anlogs incorporating fluorine and fluorine-containing groups have been synthesized using microwave irradiation and novel catalysts. The structures and physical properties and mass spectral characterization of this group of highly potent CS-type agents are described herein.

15. SUBJECT TERMS

CS	Non-lethal agents	Biochemistry	Pharmacology	Toxicology
Synthesis	Novel analogs	NMR	Mass spectral data	

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PREFACE

The work was started in May 2003 and completed in September 2004. The experimental details and the spectral data have been described in the monthly/quarterly progress reports. A portion of this work was also presented at the Bio-Defense Symposium organized by the Government of Singapore.

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CHEMISTRY, BIOCHEMISTRY, PHARMACOLOGY, AND TOXICOLOGY OF CS AND SYNTHESIS OF ITS NOVEL ANALOGS

1. INTRODUCTION

Since the first recorded use of chemicals as weapons of war around 400 B.C., man has used chemicals to manipulate the outcomes of military conflicts. However, since World War I, three groups of organic compounds (**CN, CS** and **CR**) have been used to cause temporary incapacitation and have recently found application in crowd control and management situations, though prior to this some of them were employed as chemical warfare (**CW**) agents. The latter activity has now been banned.[1] Of the three groups, two groups - the **CN** and **CR** - seem to have fallen out of grace, and the **CS**-agents have completely replaced the **CN**-agents. The **CS**-agents are currently used as riot control and anti-personnel agents in military training exercises and in testing the protective masks.

The most commonly used crowd control agent is o-chlorobenzylidene malanonitrile (**CS**).[2] Recently, **CS** was used in copious amounts by law enforcement authorities in Seattle (WA) during the meeting of the World Trade Organization to control and manage disruptive activities of the unruly crowds. The **CS**-agents are internationally used as tear gas agents. Sweden employs it under the trade name **K 62**, in military exercises and testing of the protective masks.

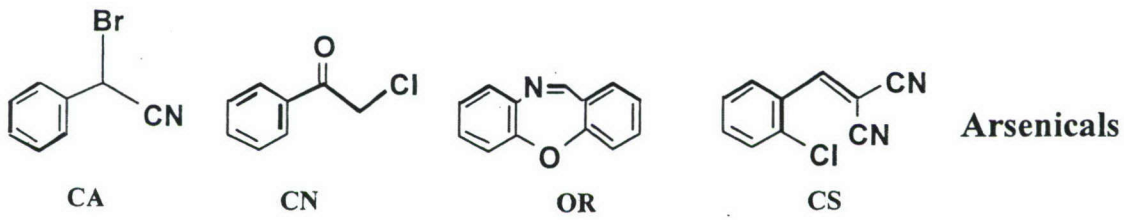


Figure 1: Five Groups of Common Crowd Management Agents

2. PHYSIOLOGICAL SYMPTOMS ARISING FROM EXPOSURE TO CS-AGENTS

In low concentration, the **CS** agents primarily act as irritants and temporary incapacitants by eliciting and inducing various physiological effects including skin irritation, copious flow of tears, running nose, coughing, dizziness, irritation of the respiratory tract, etc.[3] In high concentrations, they cause nausea and vomiting. In summary, on exposure to **CS**, one experiences (Figure 2[4]): (1) immediate sensation of skin-burning and skin reddening in warm and moist areas of the body, (2) feeling of pain, (3) watery-eyes and loss of control of the eye-lids, (4) irritation and discomfort of the respiratory tract including running nose, tingling sensation in the mouth, throat and upper respiratory tract due to its effect on the nerves of the

mucous membrane, (5) increase in blood pressure and respiration rate in animals exposed to CS aerosols, (6) acidosis and drop in the body temperature from exposures to toxic doses of the CS, (7) production of bradykinin, a vasodilator nona-peptide, in *in vivo* experiments, (8) fall in the pH, (9) sense of disorientation, loss of mental coordination and incapacitation, and (10) tapering off of the effects, in general, within 15 ~ 30 min of the termination of the exposure.

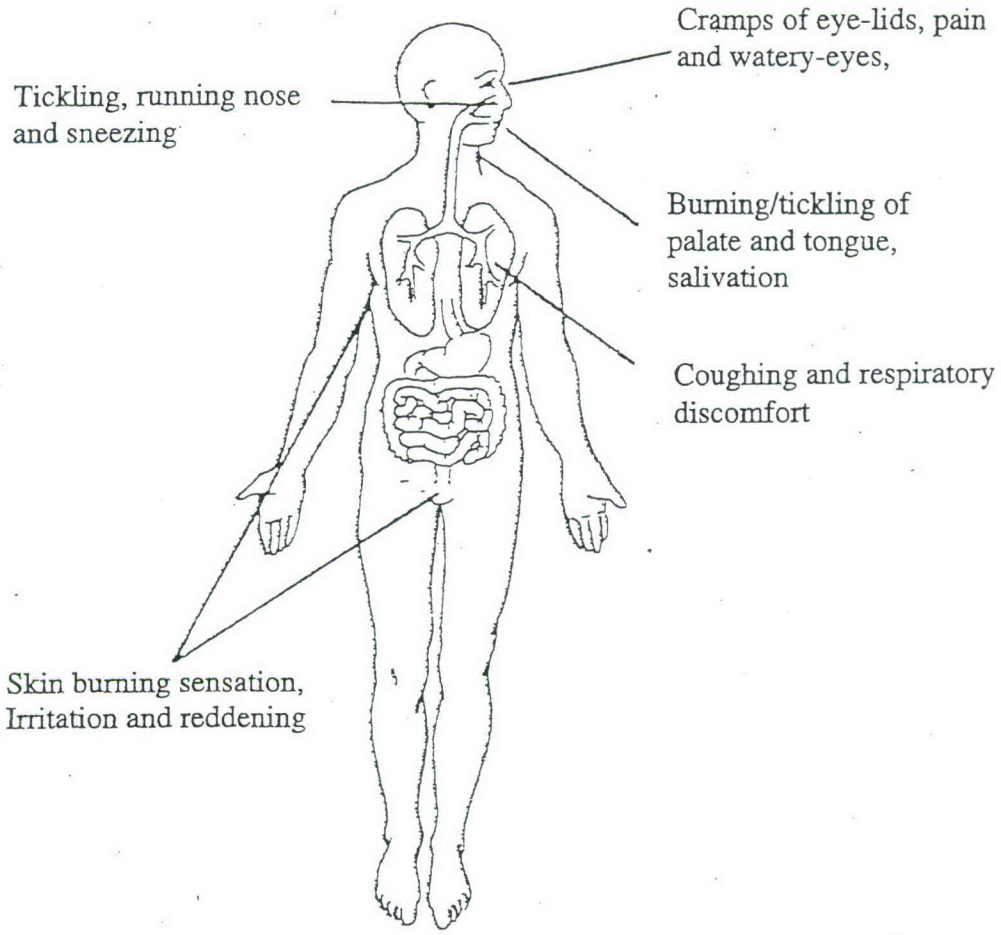


Figure 2: Symptoms Resulting from Exposure to the CS-Agents

3. SYNTHESIS OF NOVEL CS-ANALOGS

See Table 1.

4. MASS SPECTRAL BREAKDOWN

See Table 2.

Table 1: Synthesis of Novel Potential CS-Agents

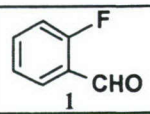
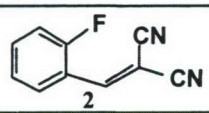
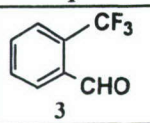
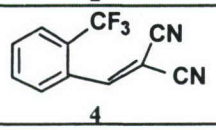
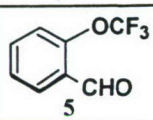
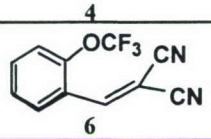
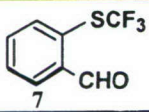
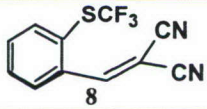
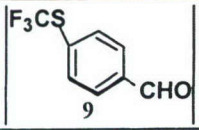
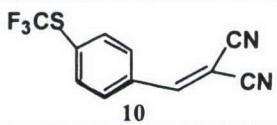
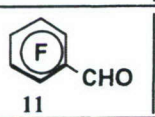
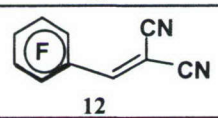
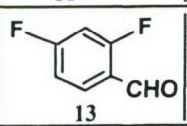
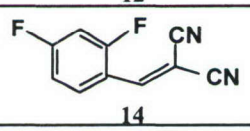
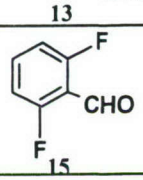
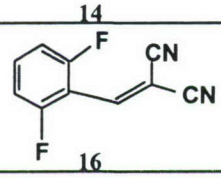
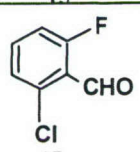
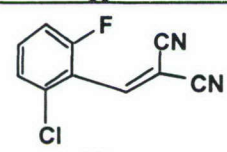
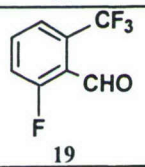
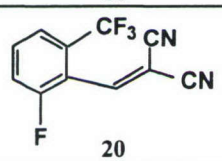
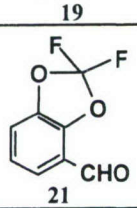
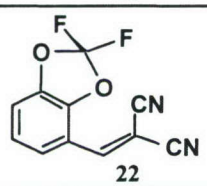
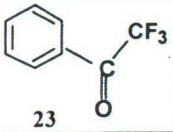
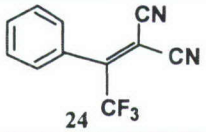
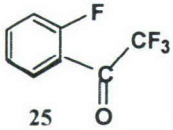
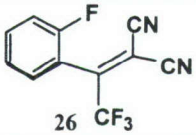
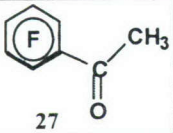
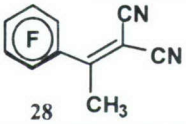
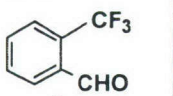
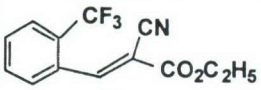
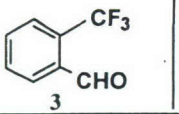
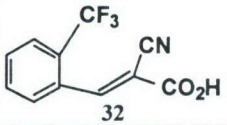
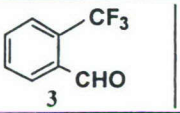
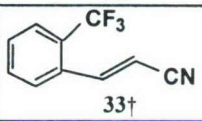
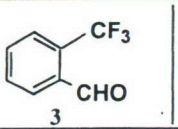
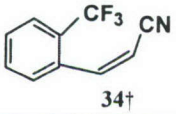
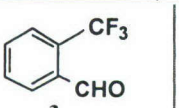
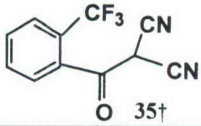
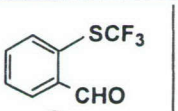
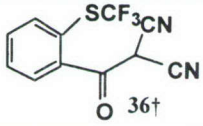
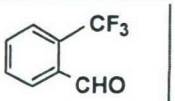
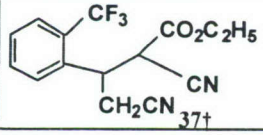
Number	Precursor	Benzylidene malononitrile	m.p. C*	Yield*+	r.t§	NMR δ; (ppm)
1	 1	 2	118 - 19°	86.4%	8.08 min	H = 8.20 F = -111.75
2	 3	 4	48 - 49°	50.0%	7.24 min	H = 8.22 F = -69.26
3	 5	 6	viscous liquid¶	86.3%	7.54 min	H = 8.18 F = -59.09
4	 7	 8	viscous liquid	87.4%	9.17 min	
5	 9	 10	66 - 67°	88.2%	10.14 min	
6	 11	 12	77 - 78°	98.9%	6.78 min	H =7.76; F = -74.2; F = -88; F = -104.21.
7	 13	 14	141 - 42°	50.8%	7.69 min	H = 8.83; F = -95.80; F = -106.20
8	 15	 16	77 - 78°	81.2%	7.96 min	H =8.85; F = -104.15; F = -104.210
9	 17	 18	80 - 81°	95.4%	9.16 min	H = 7.9 F = -103.94
10	 19	 20	92-94°	83.1%	7.06 min	
11	 21	 22	72 - 73°	95.2%	8.99 min	H = 8.01 F = 78.8

Table 1: Synthesis of Novel Potential CS-Agents (continued)

Number	Precursor	Benzylidene malononitrile	m.p.C*	Yield**	r.t.§	NMR
12	 23	 24	viscous liquid	28.5%	6.22 min	
13	 25	 26	92 - 93°	83.1%	6.28min	
14	 27	 28	viscous liquid	37.4%	6.61 min	
15	 3	 30	77 - 78°	955% ***	9.66 min	CH_3 =1.4 (t); CH_2 =34 (q); CF_3 = -59.48
16	 3	 32	165 -166°	73.0%	10.6 min	
17	 3	 33†	*	10.4%	6.06 min	
18	 3	 34†	*	4.4%	6.56 min	
19	 3	 35†	*	10.3%	6.41 min	
20	 3	 36†	*	1.1%	10.67 min	
21	 3	 37†	*	2.3%	11.42 min	

*m.p. melting points are uncorrected.
** Determined by GC-MS.
¶ viscous liquid or low melting solids at room temperature.
§ r.t.= retention time.
*** a mixture of pyridine and 4-dimethylaminopyridine was used as a catalyst.
† side products accompanying the major compound.

Table 2: Mass Spectral Fragmentation of Benzylidene Malononitriles

(1) 2-Chlorobenzylidene malononitrile (CS, r.t.=9.55 min, 98.7%): M^+=188; 161 (M - HCN); 153 (M - Cl, 100%); 137 (M - HCCCN); 126 (161- Cl); 102 (137 - Cl); 99 (126 - HCN); 75 (C_6H_3); 51 (C_4H_3) and 50 (C_3N).

(2) 2-Fluorobenzylidene malononitrile (2, r.t.=8.08 min, 86.4%): M^+=172 (99%); 152 (M - HF); 145 (M - HCN, 100%); 121 (M - HCCCN); 118 (145 - HCN); 94 (121 - HCN); 75 (C_6H_3); 63 (C_4HN); 51 (C_4H_3) and 50 (C_3N).

(3) 2-(Trifluoromethyl)benzylidene malononitrile (4, r.t.=7.24 min, 62.1 %): M^+=222 (100%); 203 (M - F); 195 (M - HCN); 183 (203 - HF); 176 (195 - F); 171 (M - HCCCN); 153 (M - CF_3); 145 (171 - CN); 126 (153 - HCN); 107 (126 - F); 99 (126 - HCN); 88 (107 - F); 75 (C_6H_3); 51 (C_4H_3 or C_3HN) and 50 (CF_2) .

(4) 2-(Trifluoromethoxy)benzylidene malononitrile (6, r.t=7..54 min, 86.3 %): M^+=238 (100%); 219 (M - F); 211 (M - HCN); 172 (219 - HF - HCN); 169 (M - CF_3); 153 (172 - F); 143 (169 - CN); 114 [$C_4H_2C(CN)_2$]; 75 (C_6H_3); 69 (CF_3); and 51 (C_3HN or C_4H_3).

(5) 2-(Trifluoromethylthio)benzylidene malononitrile (8, r.t.=9.17 min, 87.4 %): M^+=254; 235 (M - F); 215 (235 - HF); 208 (235 - HCN); 185 (M - CF_3, 100%); 158 (185 - HCN); 153 (M - SCF_3); 126 (153 - HCN);114 [$C_4H_2C(CN)_2$]; 75 (C_6H_3); 69 (CF_3); 63 (CSF) and 51 (C_4H_3).

(6) 4-(Trifluoromethylthio)benzylidene malononitrile (10, r.t.=10.14 min, 88.2 %): M^+=254 (100 %); 235 (M - F); 215 (235 - HF); 208 (235 - HCN); 185 (M - CF_3 , 97%); 158 (185 - HCN); 153 (M - SCF_3); 114 [$C_4H_2C(CN)_2$]; 100 (C_8H_4); 75 (C_6H_3); 69 (CF_3); 63 (CSF) and 50 (CF_2).

(7) Pentafluorobenzylidene malononitrile (12, r.t.=6.78 min, 98.9 %): M^+=244 (100%); 225 (M - F); 218 (M - CN); 199 (225 - CN); 193 (M - HCCCN); ; 168 (C_6HF_5); 148 (168 - HF); 130 (C_6F_3); 124 (175 - HCCCN); 93 (C_3F_3) and 69 (CF_3).

(8) 2, 4-Difluorobenzylidene malononitrile (14, r.t.=7.69 min, 50.8%): M^+=190 (100%); 169 (M - HF- H); 163 (M - HCN); 139 (M - HCCN); 124 (C_6F_2N); 101 ($C_5H_3F_2$); 88 (C_5N_2); 75 (C_6H_3); 63 (C_4HN) and 50 (C_3N);

(9) 2, 6-Difluorobenzylidene malononitrile (16, r.t.=7.96 min, 81.1%): M^+=190 (100%); 171 (M - F); 163 (M - HCN); 151 (171 - HF); 139 (M - HCCCN); 125 (C_4HNF_2); 112 ($C_6H_2F_2$); 93 (C_5NF); 75 (C_6H_3); 63 (C_4HN) and 50 (C_3N);

(10) 2-Chloro-6-fluorobenzylidene malononitrile (18, r.t.=9.16 min, 95.4%): M^+=206 [208 = ^{37}Cl]; 179 [M - CH=C(CN)$_2$]; 171 (M - Cl, 100%); 151 (171 - HF); 144 (179 - Cl); 124 (144 - HF); 118 (144 - CN); 93 (M - C_2H_2 -HCCCN); 85 (C_4H_2Cl); 75 (C_6H_3) and 50 (C_3N).

(11) 2-Fluoro-6-trifluoromethylbenzylidene malononitrile (20, r.t.=7.06 min, 82.1 %): M^+= 260 (not seen); 240 (M - HF, 100%); 221 (240 - F); 213 (240 - HCN); 194 (221 - HCN); 190 (240 - CF_2); 171 (M - CF_3); 144 (213 - CF_3); 125 (144 - F); 107 (C_6H_2NF); 99 (125 - C_2H_2); 88 ($C_4H_2F_2$); 75 (C_6H_3) and 57 (C_3H_2F).

(12) 2, 3-(Difluoromethylenedioxy)benzylidene malononitrile (22, r.t.=8.99 min, 95.4 %): M^+=234 (100%); 215 (M - F); 168 (M - F_2O_2); 171 (M - Cl, 100%); 151 (171 - HF); 144 (179 - Cl); 140 (168 - HCN - H); 113 (140 - HCN); 101 ($C_4H_2FO_2$), 75 (C_6H_3);) and 63 (C_5H_3 or (CO_2F).

Table 2: Mass Spectral Fragmentation (continued)

(13) Benzylidene α-(trifluoromethyl)malononitrile (24, r.t.=6.22 min, 28.5 %): M^+=222 (100%); 202 (M - HF); 195 (M - HCN); 183 (202 - F); 172 (M - HCCCN); 153 (M - CF_3); 126 (153 - HCN); 103 (C_6H_5CN); 88 (C_5N_2); 77 (C_6H_5); 69 (CF_3) and 51 (C_3H_3).

(14) 2-Fluorobenzylidene α-(trifluoromethyl)malononitrile (26): M^+=260 (not seen); 240 (M - HF, 100%); 221 (240 - F); 213 (240 - HCN); 194 (221 - HCN); 190 (240 - CF_2); 171 (240 - CF_3); 144 (213 - CF_3); 125 (144 - F); 99 (125 - C_2H_2); 75 (C_6H_3) and 57 (C_3H_2F).

(15) Pentafluorobenzylidene α-(methyl)malononitrile (28, r.t.=6.61 min, 37.4 %): M^+=258 (100%); 243 (M - CH_3); 238 (M - HF); 231 (M - HCN); 218 (238 - HF); 212 (231 - F); 193 (212 - F or M - HCCCN)); 181 ($C_6F_5CH_2$); 168 (C_6H_5H); 161 (181 - HF); 148 (168 - HF); 143 ($C_6F_3N_2$); 117 (143 - CN) and 93 (C_3F_3).

(16) 2-Trifluoromethyl α-carbethoxycinnamonitrile (30, r.t.=9.73 min, 95.5 %): M^+=269; 250 (M - F); 240 (M - C_2H_5); 224 (M - OC_2H_5); 200 (M - Cf_3); 196 (M - CO_2C_2H5); 176 (196 - HF); 172 ($CF_3C_6H_4C_2H_3$, 100%); 151 172 - H - HF); 126 (C_9H_5N); 99 (C_8H_3); 76(C_6H_4) and 75 (C_6H_3).

(17) 2-Trifluoromethyl β-cyanocinnamic acid (32, r.t.=6.41 min, 73.4 %): M^+=241; 224 (M - OH); 197 (M - CO_2); 176 (197 - F); 172 (M - CF_3, 100%); 169 (M - CO_2H - HCN); 128 (192 - CN - OH); 116 (C_7H_3NO); 89 (C_6H_3N); 75 (C_6H_3), 69 (CF_3) and 50 (CF_2).

(18) trans-2-Trifluoromethylcinnamonitrile (33, r.t.=6.81 min, 10.4 %): M^+=197 (100%); 177 (M - HF); 176 (177 - H); 170 (M - HCN); 158 (177 - F); 151 (151 - F); 147 (M - CF_2); 128 (149 - F); 120 (147 - HCN); 101 (128 - HCN); 75 (C_6H_3); 69 (CF_3) and 50 (CF_2).

(19) cis-2-Trifluoromethylcinnamonitrile (34, r.t.=6.95min, 4.3 %): M^+=197 (100%). The rest of the fragmentation is identical with that of its trans-isomer.

(20) 2-Trifluoromethylbenzyolmalanonitrile (35, r.t.=6.41 min, 10.8 %): M^+=238; 218 (M - HF); 210 (M - H - HCN); 190 (210 -HF); 173 [M - $CH(CN)_2$, 100%]; 151 (190 - CHCN); 145 [M - $COCH(CN)_2$]; 125 (145 - HF); 95 [$COCH(CN)_2$]; 75 (C_6H_3); 51 (C_3H_4) and 50 (CF_2).

(21) 2-Trifluoromethylthiobenzyolmalanonitrile (36, r.t.=10.67 min, 10.3 %): M^+=270;201 (M - CF_3); 185 [M - $CH(CN)_2$]; 173 (201 - HCN - H); 158 (185 - HN); 146 (173 - HCN, 100%); 126 ($C_6H_4C_2CN$); 114 (146 - S); 69 (CF_3) and 51 (C_4H_3).

(22) Ethyl (2-tifluoromethylphenyl α-cyanomethyl) cyanoacetate (37, r.t.=10.42 min, 2.3 %): M^+=310; 281 (M - C_2H_5); 265 (M - OC_2H_5); 245 (265 - HF); 237 (M - $CO_2C_2H_5$); 217 (237 - HF); 198 (M - $CHCH_2CO_2C_2H_5$, 98%); 178 (198 - HF, 100%); 156 (198 - CH_2CN); 151 [($NCCHCN(CO_2H_5)$]; 128 (198 - HCF_3); 101 (C_6H_3CN); 75 (C_5H_3) and 69 (CF_3).

5. PHARMACOLOGY AND TOXICOLOGY OF CS COMPOUNDS

The median concentration for respiratory effects is stated to be 12~20 mg/m^3, whereas for eye effects it was found to be 1~5 mg/m^3.[4] The onset time for maximum effects is 20~60 sec and the duration of time is 5~10 min after the cessation of exposure.[5] The parent compound (**CS**) exhibits LD_{50} in rats 28 mg/kg, i.v. rats 48 mg/kg and i.p. LC_{50} in rats 88 and 480mg/min/m^3.[6] Comparative toxicological studies of **CS** and **CN** have been described.[7,8] Its toxic effects have been investigated.[9] In a study of its acute toxicity, no abnormalities in EKG, respiratory function, blood biochemistry and cell constitution were observed.[10] On the other hand, the observed decrease in the respiratory rate was found to be proportional to the concentration of **CS.**[11]

The **CS**-aerosol has been reported to cause lachrymation, sternutation, sensory irritation of the upper respiratory tract, skin-sensitization, etc.[12,13] The on-set time is very rapid.[6,14] Although several toxicological studies have been conducted, it is rather difficult to accurately describe the mechanisms and effects arising from exposures to the CS vapors. One reason for this is that the ***in vivo*** **half-life of the CS has been observed to be < 30 sec**. In fact, it takes that much time to collect blood samples from the experimental animals for analysis. The ***in vitro*** half-life in blood has been reported to be 4.5 ~ 8.5 sec.[15]

Using ^{14}C-labeled **CS**, it has been conclusively demonstrated that **CS** has no effect on DNA.[16] Also, the^{14}C-labeling was not incorporated into the DNA to a significant extent. This meant that, unlike mustard, the **CS**-agents do not act as alkylating agents of the DNA. However, the ^{14}C-labeling was detected in the proteins; suggesting that either the parent **CS** or its metabolite(s) react and/or form some sort of binding with proteins. Although the exact nature of this binding has not as yet been completely characterized, it is reasonable to assume that this could be the Schiff base type linkage formed between the free amino group of the protein and the benzaldehyde moiety resulting from the biological breakdown of the **CS**. The observations of von Doniker and coworkers are supported from the report that **CS** does not exhibit any mutagenicity.[17] The **CS** compounds appear to get rapidly hydrolyzed in water to aromatic aldehydes and malononitrile.[18,19] Because of its exceptional reactivity, malononitrile has not been isolated and identified as such.

In a comparative toxicological study of **CN** and **CS**, it was found that neither showed any carcinogenic effects and **CS** did not cause any permanent damage to the eye, skin, or respiratory system.[15-20] The **CS** is reported to have a lower effective dose value, higher LD_{50} value and larger safety margin than **CN**. No dose–response relationship was observed in inhalation studies conducted in mice, rats, and guinea pigs. Ocular administration of 0.5 mL of a 10% solution of **CS** in dichloromethane produced immediate conjunctivitis, which persisted for 30 ~ 50 min and caused no permanent injury to the eyes. Within 30 sec of exposure, lachrymation and salivation occurred.

Within a few minutes after the cessation of exposure, the effects disappeared. It is important to remember that to date, no carcinogenic effects have been demonstrated from exposures to **CS**. Because it is considerably less toxic, it certainly has a distinct advantage over **CA**, **CN**, **CR**, and arsenicals. "The gross signs of **CS** intoxication are lachrimation, salivation,

lethargy and dyspnea".[20] Punte and co-workers have described a toxicological profile of the **CS** (Table 3) and the results arising from an exposure to CS aerosol.[20] Table 4 shows the threshold concentrations of **CN**, **CS**, and **CR** compounds required to produce the desired physiological symptoms.

Table 3: Toxicological Studies of 2-Chlorobenzylidene malononitrile (CS) in Rabbits and Mice

Modes of Administration	Studies	LD_{50}-values
Subcutaneous (mice)	Cumulative	800 mg/ kg
Intravenous and Subcutaneous (rabits)	Acute	8 mg/ kg

Table 4: Threshhold and Concentrantions of Non-lethal Incapacitants*

	CN	CS	CR
$[TC_{(50)}]$ (eyes)	0.3	0.004	0.004
$[TC_{(50)}]$ (respiration)	0.4	0.023	0.002
$[IC_{(50)}]$ values	20.5	3.6	0.7
* opcw.nl/chemhaz/tear.htm			

6. BIOLOGICAL FATE, MECHANISM OF ACTION AND METABOLISM OF CS

The **CS** has been stated to be environmentally non-persistent, which means it is rapidly metabolized and broken down.[6] However, its *in vivo* metabolic fate seems to depend on its mode of entry into an animal. The fate of the aerosol adminstrered **CS** is somewhat erratic. When administered inrtravenously, its metabolism is smooth and is found to rapidly bind with the plasma proteins to form **CS**-protein complexes.[20] Its breakdown begins as soon as it enters the system. After 50 sec, it can be detected in the blood, which happens to be the most active metabolic site of **CS**, rather than any specific tissue.[20] The plasma bound **CS** may be antigenic.[21] Inhalation and dermal absorption are the only processes through which the **CS** can enter into the body. The effects of inhalation are rapid and manifest within a very short time. The manifestation of the effects of dermal absorption takes some time. First, the **CS** has to be absorbed through the skin and then must reach the nervous system before it can exert its effects.

Lethal and non-lethal chemical agents bring about their effects by interfering with the transmission of nerve impulses either via blocking the transmission or by over flooding the neural system with too many messages to transmit and hence confusing the neural system. This process takes time.

Figure 3 describes the various steps involved in the metabloism of the **CS**.[22] Two pathways have been advanced to explain the metbolism of **CS**. The first pathway involves the reduction of the exocyclic double bond to form benzyl malonnoitrile. The second pathway consists of the retro-Knovenagel type reaction to regenerate the aromatic aldehyde derivative. The benzyl malonnoitrile, thus formed, has been stated to give rise to malononitrile, which immediately breaks down to **CN** ion, and in turn is converted into thiocyanate. The aromatic aldehyde formed in the scecond pathway has two options open to it. The first option is for the aromatic aldehyde to become oxidized to benzoic acid. The latter then yields hippuric acid by reacting with glycine. Second option, the aldehyde becomes reduced to benztyl alcohol, which forms mercapturic acid derivative via the sulfonate ester intermediate. The 2-Chlorobenzylmalononitrile has been identfied as a metabolite of **CS**.[23] Thiocyanate has also been detected after intravenous administration of **CS**.[14] This lends credence to the proposed pathways. The **CS** has been observed to react with the thiol moiety at neutral pH.[24] Very little or no work has been reported with purified enzyme systems. The **CS** has been reported to affect the activity of a host of enzymes and also to suppress the activity of esterases.[25]

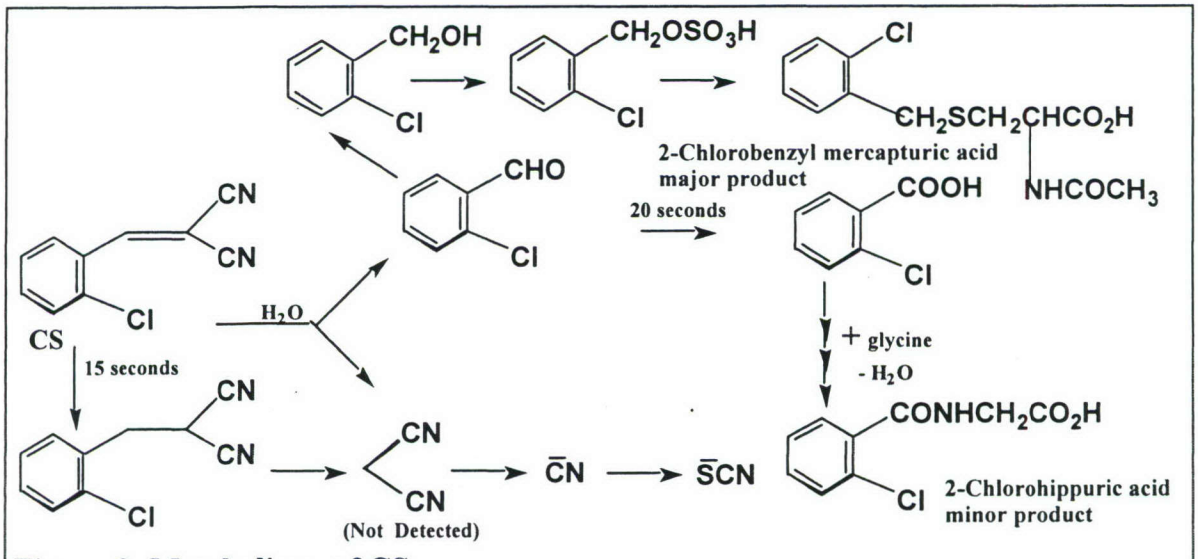


Figure 3: Metabolism of CS

7. MEDICAL APPLICATIONS

Although positive results were obtained in treating malignant tumors in animals with **CS**,[26,27] studies with human patients suffering from advanced sarcoma and carcinoma were found to be not encouraging.[28] "No severe reactions were observed. There were no accute effects on pulse, respiration, temperature, blood pressure, or electrocardiographic patterns. In none of the patients was there an observable effect upon primary tumor or its metastases.

No toxic effects were seen and none of the patients derived subjective or objective clinical improvement".[29]

8. METHODS OF DETECTION OF CS

Several methods have been developed and described for the detection of **CS**.[30] Benzofurane oxide is reported to give a distinct violet color with **CS**. A bioassay screening test has been reported,[31] which essentially consists of applying the sample to the skin and measuring the activity of non-specific esterases using a computer generated image analysis procedure. A quick and dirty method of thin layer chroamtographic[32] and gas chromatographic techniques[30,33] have been used in the detection of these compounds.

9. PROJECT OBJECTIVE

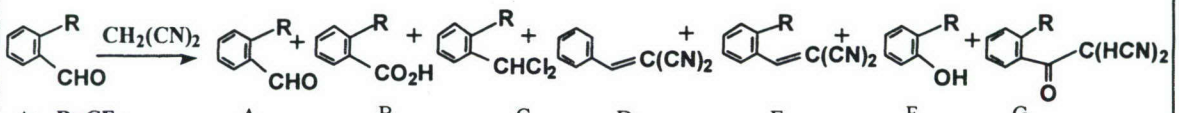
The primary objective of this project was to synthesize environmentally benign, safe, and biologically more potent **CS** analogs. To this end, the synthesis of a novel group of **CS**-agents incorporating fluorine and fluorine containing groups has been accomplished under microwave irradiation using new catalysts. This report describes the synthesis and physical properties of a highly potent group of new **CS**-type compounds (Table 1) and their mass spectral fragmentation behavior (Table 2).

10. RESULTS AND DISCUSSION

Recently benzylidene malononitriles have attracted considerable attention for use as non-lethal crowd control and law enforcement chemical agents. Alkylidenation of the carbonyl compounds via the classical Knovenagel condensation is of general and wide application for creating a carbon-carbon double bond. In general, this reaction is catalyzed by bases or ammonium salts[34]. Recently a variety of catalysts such as $TiCl_4$,[35,36] CdI_2,[37] alumina,[38,39] xeolites,[40,41] siilylated-amines,[42] silica;[43,44] silica functionalized amines[45] and Al_3PO_4-Al_2O_3 complex[46] have found application in the Knovnagel reaction. Solvent free microwave reactions have also been employed for the synthesis of these compounds.[47] Instead of the carbonyl precursors, imines have been used and found to give similar results.[48] Even amino acids have been stated to catalyze this condensation.[49] The Knovenagel condensation has been carried out diastereoselectively[50,51] and enantioselectively.[52,53] Dilithio N-methanesulfinyl-p-toluidine,[54] tosylmethylisocyanide[55], α-methoxyvinyllithium,[56] lithiated allylic carbamate,[57] and lithium bis(ethylenedioxyboryl)methide[58] have been employed as catalysts in the modified version of this reaction. Recently, antimony based *in situ* generated catalysts have been used to successfully alkylidenate steroidal ketones.[59] The reaction has been shown to occur in the presence of LiBr.[60] From the above narrative, it seems that almost any reagent can catalyze the Knovenagel reaction. Two mechanisms have been advanced to explain and rationalize the formation of the reaction products.[34] Based on the observation that organic bases catalyze the reaction, Hann and Lapworth suggested that the organic bases generate carbanions from the reactive methylene group, which subsequently reacts with the carbonyl compounds to

furnish β-hydroxyl compounds,[34] which then undergo facile dehydration during the course of the reaction to yield the end products.

Table 5: Product Distribution with Different Catalysts



A: R=CF_3

	$CH_2(CN)_2$	A	B	C	D	E	F	G
Activated alumina-bead powder	11.4%	27.8%	1.5%	0.3%*		49.0%		
Europium (III) fluoride	2.2%	3.7%	2.9%	0.7%		90.0%		
Hexafluoroammonium aluminate**	5.9%	19.5%				62.0%	0.6%	10.8%
Piperidine		37.0%	4.0%		0.4%	56.6%		
No catalyst		81.6%	0.4%			8.0%		
Pyridine		1.5%		1.2%	6.7%	88.4%		

* impurity present in the starting material, o-(trifluoromethyl)benzaldehye.
** Two additional compounds were characterized from this reaction product, namely 2-(trifluoromethyl)-phenol (0.6%) and 2-(trifluoromethylbenzoyl malononitrile(10.8%).

With a view to developing synthetic methodology, microwave assisted synthesis of benzylidene malononitriles in the neat phase has been explored using new catalysts such as europium (lll) fluoride, hexafluoroammonium aluminate, freshly activated alumina-pellet (3 mm) powder, and 1,2,2,6,6-pentamethyl-piperidine. The mass spectral fragmentation and nuclear magnetic resonance (NMR) characteristics of 15 compounds thus synthesized are given in Tables 2, 3, and 6. What is unique about the present work is the characterization of 2-(trifluoromethyl)- and 2-(trifluoromethylthio)benzoyl malononitriles. This suggests that initially formed β-hydroxy intermediates get oxidized during the course of the reaction to furnish the above cited benzoyl malononitriles. To the best of our information, this has not been so far observed in the Knovenagel reaction. Of the 15 compounds, only one compound, namely 6 (Table 1) has a slightly higher retention time than that of **CS**. The remaining 14 compounds should, in principle, possess lower vapor pressure than compared to that of **CS**. The microwave process described herein possesses a distinct advantage. The reactions can be carried without the use of any solvent and thus, avoid the generation and accumulation of hazardous laboratory waste. In addition, the reaction times are dramatically reduced, and the formation of unwanted side-products minimized.

Four new catalysts, [hexafluoroammoniumaluminate, europiun (III) fluoride, pentamethylpiperidine, and freshly activated alumina-pellet powder 3 mm] were used in the exploratory work with 2-trifluoromethylbenzaldehyde and malononitrile as reactants. However, in the latter work, only freshly activated alumina-pellet powder was used as a catalyst to avoid the formation of the side-products. In the EuF_3 catalyzed reaction of

2-fluoromethylbenzaldehyde with malononitrile, the presence of 2-trifluoromethylbenzoyl malononitrile (**35**, 0.2%) was detected by gas chromatography mass spectrometry (GC-MS). The identity of the compound was confirmed by the presence of the $[COCH(CN)_2\}$ ion (m/e=51) in its mass spectrum. Also, the characterization of 2-trifluoromethylbenzoyl malononitrile (**36**, 10.3%) in the Knovenagel reaction product formed with hexafluoroammoiumaluminate as catalyst suggests that the initially formed β-hydroxy derivative had undergone *in situ* oxidation.[61] The origin of 2-trifluorphenol is not yet known with certainly, although it could have been formed by the Baeyer-Villiger type oxidation of the substrate during the course of the reaction.

The routine work-up and chromatography over silica of the reaction product of the condensation of 2-trifluoromethylbenzaldehyde with ethyl cyano-actetate in the presence of pyridine and 4-dimethylaminopyridine furnished trans-2-trifluoromethylcinnamonitrile (1.1%) and ethyl 3-(2-trifluoromethylphenyl)-3-(cyanomethyl)-2-cyanopropionate (**37**, 2.3%) along with the expected product (**30**, see Table 1). The formation of **37** must have involved the Michael-type base catalyzed addition of ethyl cyanoactetate to the cinnamonitrile derivative, also formed during the reaction (see Table 1). This inference is supported by the observation that acrylonitrile derivatives are known to serve as substrates in the formation of the carbon-carbon bond during the stepwise Michael type addition reaction.[62] The characterization of ethyl 3-(2-trifluoromethyl-phenyl-3-cyanomethyl)-2-cyanoacetate as one of the products of the Knovenagel reaction of 2-trifluoromethylbenzaldehyde with ethyl cyanoacetate was somewhat unexpected, although its genesis can be traced to the Michael type addition of ethyl cyanoactate to trans-2-trifluoromethylcinnamonitrile in the presence of basic catalysts. The latter precursor was generated during the course of the reaction. This inference was confirmed by the GC-MS characterization of 2-trifluoromethyl-cinnamonitrile as one of the products of the same reaction. Additional support comes from the recent observation that the ultrasound catalyzed addition of reactive methylene containing compounds to acrylonitriles furnishes the β-adducts.[63]

10.1 Mass Spectral Fragmentation Mechanism.

Mass spectral fragmentation behavior of benzylidene malononitriles has been discussed briefly.[64] The primary breakdown process from the parent ion appears to be the loss of hydrogen cyanide (HCN). Also seen is the elimination of propargyl nitrile (HCCCN) moiety from the parent ion generated from benzylidene malononitrile to yield C_6H_5CN radical cation (m/e= 103) in a one-step process (Figure 4). New bond formation reactions during the electronic bombardment have been observed.[65] A similar fragmentation behavior is observed during the mass spectral breakdown of the compounds described in this report. The loss of HCN and (HCCCN) fragments (m/e= 27 and 51) was characteristically observed (Figure 4 and Table 2). The facile loss of F from the molecular ions was also seen in most of the compounds.

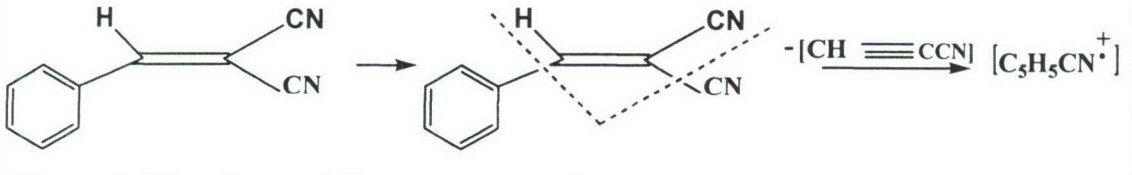


Figure 4: Mass Spectral Fragmentation of Benzylidene Malononitrile

10.2 NMR Data.

The NMR spectra of the benzylidene malononitrile have been described.[66] The chemical shift of the α-proton of the CS-type compounds appears to vary from 7.8 to 8.8 ppm, depending on the nature and position of the substituent on the aromatic ring. Thus, the signal due to ^{1}H (α-proton of **CS**) appears at 8.51 ppm in DMSO,[66] while Mesilaakso observed it at 8.26 ppm in $CDCl_3$.[67] The α-proton has been shown to couple with the ring-hydrogens,[67] and its chemical shifts depend on the solvents and the nature of substituents on the aromatic ring.[67] The ^{13}C-signal due to $\mathbf{C}(CN)_2$ was reported to be at 8.52 ppm. The NMR data described herein is similar to this value except in one case, namely that of entry # 6. In view of the reported correlation between the substituent constants and MNR absorption, the above stated changes in the chemical shifts appear to be reasonable and acceptable.[65] Table 1 gives ^{1}H and ^{19}F chemical shifts, while Table 6 describes the ^{13}C-NMR data.

11. CONCLUSION

The new **CS**-analogs are expected to be more potent than **CS**. This observation is based on the following considerations. First, fluorine is biologically more potent than chlorine. Second, the presence of fluorine makes the new compounds more volatile than **CS**, and hence, they should all possess lower vapor pressure than that of **CS**.[68] This inference stands supported by the fact that they all exhibit lower gas chromatographic retention times than the **CS** does (Table 1). Because the carbon-fluorine bond is one of the strongest bonds, unlike **CS**, which contains chlorine, the new compounds ought to be environmentally benign. The presence of fluorine in the new compounds permits their *in vivo* biological screening with PET (Positron Emission Tomography). Thus, the new compounds possess several distinct advantages over **CS**.

12. EXPERIMENTAL PART

The organofluorine compounds used as substrates in this work were procured from SynQuest Fluorochemicals, Oakwood Research Chemicals and JRD Fluorochemicals, Ltd. (England). Fluorine-containing aldehydes are highly irritating compounds. Care and caution should be exercised to avoid direct exposure to eyes and skin. The alumina pellets (3 mm, Aldrich Chemical Company) were activated by heating at 120 - 130 °C in a heated vacuum oven, cooled to room temperature and freshly ground powder was used immediately. Stoichiometric amounts of the respective reagents were mixed in glass vials or 5 mL ground joint round bottom flasks and stoppered, vigorously shaken on a vibro-mixer and heated in the desktop kitchen Panasonic microwave oven for specified periods of time. The reaction mixture was allowed to come to ambient temperature, taken up in dichloromethane, and filtered over cotton-wool, then first analyzed by gas chromatography and finally was subjected to the GC-MS analysis.

Table 6: ^{13}C-NMR Chemical Shifts of Substituted Benzylidene malononitriles

Entry 1*: **C**F (163.33); **C** (ring) CH=C (CN) $_2$ (126.30); **C**N (119.45); **C**N (119.29): **C**H=C (CN) $_2$ (159.21); CH= **C** (CN) $_2$ (81.89).

Entry 2: CF_3 (210. 15); **C** (ring) CH=C(CN) $_2$ (132.08); **C**N (113.13); **C**N (111.4): **C**H=C (CN) $_2$ (156.69); CH= **C** (CN) $_2$ (85.62)

Entry 3: OCF_3 (120. 62); **C** (ring)CH=C(CN) $_2$ (132.51); **C**N (112.51); **C**N (113.85): **C**H=C (CN) $_2$ (**153.62**); CH= **C** (CN) $_2$ (86.05)

Entry 4: SCF_3 (117. 14); **C** (ring)CH=C(CN) $_2$ (132.08); **C**N (117.20); **C**N (117.28): **C**H=C (CN) $_2$ (164.25); CH= **C** (CN) $_2$ (78.29)

Entry 6: ; **C**N (111.95); **C**N (116.24): **C**H=C (CN) $_2$ (142.95); CH=**C** (CN) $_2$ (**93.82**)

Entry 7: **C**F (161.4); **C**F (162.0); **C**N (113.56); **C**N (112.2): **C**H=C (CN) $_2$ (159.03); CH= **C** (CN) $_2$ (78.5)

Entry 8: **C**N (113.56); **C**N (111.21): **C**H=C (CN) $_2$ (159.03); CH=**C** (CN) $_2$ (88.1)

Entry 11: CF_2 (111.85); **C**N (112.84); **C**N (114.831): **C**H=C (CN) $_2$ (**149.45**); CH= **C** (CN) $_2$ (**86.78**)

Entry 12: **C**F (136.21); **C**Cl (130.60); **C**N (112.22); **C**N (112.31): **C**H=C (CN) $_2$ (155.98); CH=**C** (CN) $_2$ (88.1)

CS: **C** (ring)CH=C(CN) $_2$ (130.6); **C**Cl (136.22); **C**N (113.13); **C**N (111.82): **C**H=C (CN) $_2$ (155.9); CH= **C** (CN) $_2$ (85.6)

* Refers to the serial number in **Table 1**.

Mass spectra were obtained using a Finnigan TSQ-7000 GC/MS/MS equipped with a 30 m x 0.25 mm. i.d. Rtx-5 ms capillary column (Restek, Bellefonte, PA). The conditions on the TSQ-7000 were: oven temperature 60-270 °C at 15 °C/min, injection temperature 220 °C, interface temperature 250 °C, source temperature 150 °C, electron energy 70 eV (EI) or 200 eV (CI), emission current 400 μA (EI) or 300 μA (CI), and scan time 0.7 sec. Data was obtained in the electron ionization mode (range 45-450 da) and chemical ionization mode (mass range 60-450 da). Ultrahigh purity methane was used as the CI agent gas with a source pressure of 3.5 Torr (TSQ-7100). Routine GC analyses were accomplished with a Varian 3400 GC equipped with a J and W Scientific (Folsom, CA)30 m x 0.53 mm i.d. DB-5 column. Routine GC analyses were accomplished with a Varian 3400 GC equipped with a J and W Scientific 30 m x 0.53 mm i.d. DB-5 column (J and W Scientific, Folsom, CA). The NMR spectra (^{1}H and ^{13}C) were recorded in $CDCl_3$ with TMS as the internal standard on a Varian VXR-400S spectrometer at 100 MHz and 376 MHz, respectively. The external reference for ^{19}F was CCl_3F. The melting points were recorded using a Thomas Hoover Capillary melting point apparatus and are uncorrected.

A total of 15 fluorine containing novel **CS**-compounds were synthesized using the general procedure (cf. entries 1 through 14, Table 1). In addition, 2 substituted cinnamic acid derivatives, 2 substituted benzoyl malononitriles, and 2 cinnamonitriles have been identified.

Because our primary objective was to synthesize novel analogs of **CS**, the above mentioned side-products were not isolated.

12.1 Synthesis of 2-Trifluoromethylbenzylidene Malononitrile (**4**) (General Procedure for Activated Alumina-Bead Powder Catalyzed Preparation).

A mixture of stoichiometric amounts of 2-trifluoromethylbenzaldehyde (**3**, 43 mg) and malononitrile (23 mg) and catalytic amount alumina oxide catalyst (20 mg) in a small vial was exposed to microwave radiation for 2.5 min. The reaction mixture was cooled to room temperature, treated with a small amount of dichloromethane, filtered to remove the catalyst and analyzed by GC and then by GC-MS. The above reaction was repeated using different catalysts such as europium (lll) fluoride, hexafluoroammonium aluminate and 1,2,2,6,6-pentamethylpiperidine. A similar reaction was carried out under identical conditions except that no catalyst was used in the condensation. Less than 8% of expected product was obtained and the starting material constituted the rest. Table 5 describes the formation and distribution of the various compounds.

12.2 Condensation of 2-Trifluoromethylbenzaldehyde **(3)** with Cyanoacetic Acid.

Pyridine (0.2 mL) was added to a mixture of 2-trifluoromethylbenzaldehyde (**3**, 0.348 g, 2 mmol) and cyanoacetic acid (0.170 g, 2 mmol), and the whole was shaken with the aid of a vibromixture for 2 min and initially exposed to microwave irradiation for 2 min. Then it was heated for another 2 min. The cooled mixture was processed as usual and the product on GC-MS analysis was found to contain (1) trans-2-trifluoromethylcinnamonitrile (**33**, 16.4%); cis-2-trifluoromethylcinnamonitrile (**34**, 4.3%) and β-(2-trifluoromethylphenyl)-α-(cyano) acrylic acid (**32**, 73.0%) along with 2-trifluoromethylbenzaldehyde (**3**, 1.5%) and 2-trifluoromethyl-benzalchloride (1.2%). After the solvent was evaporated under reduced pressure, the residue was chromatographed over silica. Elution with petroleum ether removed the last two compounds. The first two compounds were obtained with pet. ether-ether (90:10). The desired compound came out with pet. ether -ether (70:30).

12.3 Condensation of 2-Trifluoromethylbenzaldehyde **(3)** with Ethyl Cyanoacetate.

Pyridine (0.1 mL, and 4-dimethylaminopyridine (100 mg) was added to a mixture of 2-trifluoromethylbenzaldehyde (**3**, 0.348 g., 2 mmol) and ethyl cyanoacetate (0.226 g., 2 mmol) and was shaken with the aid of a vibromixture for 2 min and heated in a desktop kitchen microwave oven for 4 min. The mixture was cooled to ambient temperature, extracted with methylene chloride (20 mL) and processed as usual. After the solvent evaporated, the residue was chromatographed over silica to yield the product (0.431 g). The GC-MS analysis of the residue showed the presence of three components: (1) trans-2-trifluoromethylcinnamonitrile (**33**, 1.1%); (2) ethyl β-(2-trifluoromethylphenyl)-α-cyanoacrylate (**30**, 95.5%); and (3) ethyl β-(2-trifluoromethylphenyl)-β-(cyanomethyl)-α-cyanopropionate (**38**, 2.3%).

12.4 Condensation of 2-Trifluoromethylthiobenzaldehyde (**7**) with Malononitrile.

Europeum (III) fluoride (0.100 g) was added to a stoichiometric mixture of 2-trifluoromethylthiobenzaldehyde (**7**, 0.412 g, 2 mmol) and malononitrile (0.132 g, 2 mmol), and the mixture was thoroughly shaken on a vibromixture for 2 ~ 3 min and heated in a desktop microwave oven for 4 min. The mixture after cooling to room temperature was treated with dichloromethane (10 mL), the organic layer was filtered through cotton-wool and the filtrate on GC-MS analysis showed the presence of seven components: (1) benzaldehyde (1.8%); (2) benzyl alcohol (0.9%); (3) 2-trifluoromethylthiobenzaldehyde (**7**, 55.5%); (4) 2-trifluoromethylthiobenzoic acid (0.1%); (5) 2-trifluoromethylthiobenzylidene malononitrile (**8**, 35.1%); (6) 2-trifluoromethylthiobenzoyl malononitrile (**36**, 0.2%); and (7) an unknown compound (0.2%). The benzaldehyde and benzyl alcohol derivatives mentioned above, were not present in the substrate, as shown by the GC-MS analysis of the substrate and hence must have been formed during the course of the reaction. The 2-trifluoromethylthiobenzoic acid obviously resulted from the oxidation of the substrate itself. It is interesting to note the formation of 2-trifluoromethylthiobenzoyl malononitrile (**36**). The last component coming off the column could not be identified as it underwent extensive fragmentation in the ion source.

12.5 Condensation of Pentafluoroacetophenone (**27**) with Malononitrile.

A stoichiometric mixture of pentafluoroacetophenone (**27**), malononitrile and 1,2,2,6,6-pentamethylpiperidine was reacted as before. The routine processing of the reaction product showed the presence of two compounds: (1) pentafluoroacetophenone (**27**, 62.5%) and (2) pentafluorobenzylidene malononitrile (**28**, 37.4%). The mixture was separated by chromatography over silica.

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